CSIRO PUBLISHING
www.publish.csiro.au/journals/ajar *Australian Journal of Agricultural Research*, 2007, **58**, 498–506

Cytogenetics in the age of molecular genetics

Peng Zhang[A,C], *Bernd Friebe*[B], *Bikram Gill*[B], *and R. F. Park*[A]

[A]Plant Breeding Institute, University of Sydney, 107 Cobbitty Road, Camden, NSW 2570, Australia.
[B]Wheat Genetics Resource Center, Department of Plant Pathology, 4024 Throckmorton Plant Sciences Center, Kansas State University, Manhattan, KS 66506, USA.
[C]Corresponding author. Email: pengzhang@camden.usyd.edu.au

Abstract. From the beginning of the 20th Century, we have seen tremendous advances in knowledge and understanding in almost all biological disciplines, including genetics, molecular biology, structural and functional genomics, and biochemistry. Among these advances, cytogenetics has played an important role. This paper details some of the important milestones of modern cytogenetics. Included are the historical role of cytogenetics in genetic studies in general and the genetics stocks produced using cytogenetic techniques. The basic biological questions cytogenetics can address and the important role and practical applications of cytogenetics in applied sciences, such as in agriculture and in breeding for disease resistance in cereals, are also discussed. The goal of this paper is to show that cytogenetics remains important in the age of molecular genetics, because it is inseparable from overall genome analysis. Cytogenetics complements studies in other disciplines within the field of biology and provides the basis for linking genetics, molecular biology and genomics research.

Additional keywords: chromosome banding, fluorescence *in situ* hybridisation (FISH), deletion lines, physical mapping, chromosome landmark, alien gene introgression.

Introduction

By the beginning of the 20th Century, Boveri and Sutton, cytologists from Germany and the USA, respectively, had fully established the chromosomal basis of Mendelian inheritance and the chromosome theory of heredity, which showed that there was a correlation between gene transmission and chromosome behaviour (Boveri 1902; Sutton 1903). Cytology and genetics subsequently had a close relationship, with the results in one field strongly affecting the other. For instance, cytogenetic studies by Morgan *et al*. (1915) in drosophila established the fruit fly as a major genetic model, especially with the discovery of polytene chromosomes by Painter (1933). Drosophila continues to be a genetic model; the complete genome was sequenced in 2000 (Adams *et al*. 2000). With the discovery of DNA structure in the 1950s (Watson and Crick 1953), molecular genetics bloomed. Cytogenetic techniques were combined with molecular techniques and new information on how DNA is arranged in chromosomes, how chromosomes replicate, and how they determine faithful transmission of genetic information in cell division was discovered. Between 1950 and 1970, cytogenetics was stagnant because there were few advances in new techniques. There were suggestions that 'cytogenetics is a dead science' and that it was no longer needed because of advances in other disciplines of biology. However, even during this period, many advances were made in the cytogenetic mapping of crop plants. These studies involved organisms such as wheat and provided the basis on which wheat was established as an important model for cytogenetic studies of allopolyploids.

Using traditional cytogenetic techniques, Sears developed many valuable wheat genetic stocks (Sears 1954, 1966*a*), such as full sets of monosomics, ditelosomics, double ditelosomics, and nullisomic–tetrasomics, which are still used widely and continue to be important for both cytogenetic and genetic studies in wheat. The wheat deletion lines developed by Endo and Gill (1996) provide a unique way to conduct physical mapping in wheat and also are valuable materials for molecular genetics studies.

The application of biochemical and molecular biological developments to cytogenetics led to the development of chromosome banding (Gill and Kimber 1974*a*, 1974*b*) and fluorescence *in situ* hybridisation (FISH) techniques (Rayburn and Gill 1985) in wheat, which have revolutionised cytogenetic studies. These tools allowed the study of the structure and function of chromosome landmarks such as heterochromatin (Bedbrook *et al*. 1980; Appels *et al*. 1981), rRNA genes (Mukai *et al*. 1990; Jiang and Gill 1994*b*), centromeres (Zhang *et al*. 2001), subtelomeres (Zhang *et al*. 2004*a*), and telomeres (Friebe *et al*. 2001), and also the evolution of wheat and other species in the Triticeae family (Naranjo *et al*. 1987; Jiang and Gill 1994*c*; Schmidt and Heslop-Harrison 1996). In addition, cytogenetics plays a vital role in genome sequencing and in the study of chromosome structure and function. Combined cytogenetic and molecular approaches have greatly advanced wheat genome analysis.

In order to reduce the risk of genetic erosion and to broaden the genetic diversity in cultivated wheat, agronomically interesting genes have been introgressed into wheat from progenitor and wild relatives (Jiang *et al*. 1994; McIntosh

 10.1071/AR07054 0004-9409/07/060498

et al. 1995; Friebe *et al*. 1996). Relying heavily on cytogenetic tools, new sources of disease resistance were identified and many resistance genes, including rust resistance genes, were introgressed from alien species. To date, 23 leaf rust, 19 stem rust, and 6 stripe rust resistance genes have been transferred to wheat from alien species (Jiang *et al*. 1994; McIntosh *et al*. 1995, 2003; Friebe *et al*. 1996). Cytogenetic tools and chromosome engineering will continue to be useful in facilitating the transfer of further rust resistance genes from alien species, providing new sources of disease resistance, reducing linkage drag, pyramiding resistance genes, and breeding for durable resistance. Results from our own work and from other researchers indicate that we still need cytogenetics in the age of molecular genetics.

A century of cytogenetics research

Many important advances were made in the last century in various disciplines of biology. Among these advances, cytogenetics has developed dramatically and is used in many different areas of research. Boveri and Sutton both studied the cytology of meiosis and independently showed that chromosome behaviour mimics Mendel's law. They concluded that chromosomes must be the carriers of genetic information, which we now know are genes, and fully established the chromosomal basis of Mendelian inheritance (Boveri 1902; Sutton 1903). The last century can be divided into 2 parts, the first (1900–1949) the 'age of classical cytogenetics'. With the discovery of DNA structure by Watson and Crick (1953), molecular genetics and cytogenetics bloomed. Scientists began to know that DNA is organised into chromosomes. The second half (1950–present) is 'the age of molecular cytogenetics'. Between 1950 and 1970, many advances were made in cytogenetic mapping in crop plants, including wheat. Major conceptual advances in polyploidy cytogenetics also occurred, using wheat as an allopolyploid inheritance model and potato as an autopolyploid model.

From the 1970s there were many advances in cytogenetic and molecular cytogenetic techniques, such as the different chromosome banding techniques (Gill and Kimber 1974*a*, 1974*b*), *in situ* hybridisation (ISH) (Gall and Pardue 1969), microscopy, and DNA manipulation. This propelled the second cytogenetics revolution. Different types of banding techniques allowed the identification of individual chromosomes and a revolution in plant cytogenetic identification began because chromosomes of virtually all organisms could be fingerprinted. These banding techniques are still used routinely in human disease diagnosis and plant research. Among the many plant species, wheat was a major beneficiary of chromosome banding techniques and the molecular cytogenetics revolution. C-banding in wheat has been used to analyse the substructure of wheat chromosomes. It not only allows fast and reliable identification of all 21 chromosomes but also permits the identification of 38 of the 42 chromosome arms (Gill *et al*. 1991).

FISH was originally derived from the ISH technique, which used isotopes to label probes to detect the DNA or RNA sequences in cytological preparations (Gall and Pardue 1969). In 1982, a non-radioactive (immunological) FISH method using fluorochromes for signal detection was developed (Langer-Safer *et al*. 1982) and, since then, has been used widely in different areas of human, animal, and plant research (Jiang and Gill 1994*a*) as well as in routine clinical diagnoses. Using the FISH technique, DNA sequences such as repetitive DNA sequences (Langer-Safer *et al*. 1982; Rayburn and Gill 1985), multi-copy gene families (Mukai *et al*. 1990), and low- or single-copy genes (Langer-Safer *et al*. 1982; Viegas-Pequignot *et al*. 1991; Leitch and Heslop-Harrison 1993) can be physically localised directly on chromosomes. FISH has also been applied in chromosome identification and molecular karyotype construction (Rayburn and Gill 1985; Mukai *et al*. 1993; Pedersen and Langridge 1997; Zhang *et al*. 2004*b*). FISH using bacterial artificial chromosomes (BAC) as probes has been used to verify the quality of BAC libraries constructed in different organisms (Woo *et al*. 1994). In the 1990s, the even more powerful technique of fibre-FISH, using extended DNA fibres as targets for FISH, was developed (Fransz *et al*. 1996). Fibre-FISH greatly improved the sensitivity and resolution of the FISH technique and allowed the mapping of probes that are 1 kb to 1 Mb apart (Jackson *et al*. 1998). It helped to estimate the size of the gap between rice BAC clones, which was difficult to achieve by other techniques, closed the gap, and facilitated the rice genome sequencing project (J. Jiang, pers. comm.). Combining the FISH technique on different DNA targets allows us to map DNA sequences on well-differentiated chromosomes at a higher resolution. Because of its specificity, clarity, and relative rapidity of detection, FISH remains the technique of choice for direct visualisation of genomes, chromosomes, chromosome segments, genes, DNA sequences, and their order and orientation. Unlike many other classical techniques that plateau or decline in popularity as new technologies displace them, FISH is more powerful after more than 25 years and continues to make important contributions in areas such as genomic structure and gene expression studies. No wonder that Eisenstein (2005) has titled one of his articles, 'A look back: FISH still fresh after 25 years'. In addition to the FISH technique, chromosome banding techniques and classical meiotic pairing analysis are still important tools and the basis for many molecular genetics, molecular biology, and genomics studies. For example, analysis of human–hamster somatic cell hybrid chromosomes by banding and FISH (Pinkel *et al*. 1986) was critical in human chromosome mapping and led to the sequencing of the first human chromosome in 1999 (Dunham *et al*. 1999) and soon after the sequencing of other human chromosomes.

Genomic *in situ* hybridisation (GISH) (Pinkel *et al*. 1986; Le *et al*. 1989), a special type of FISH that uses genomic DNA of a donor species as a probe in combination with an excess amount of unlabelled blocking DNA, provides a powerful technique to monitor chromatin introgression during interspecific hybridisation. In addition, the GISH technique allows the study of genome affinity between polyploidy species and their progenitors. GISH is thus a valuable supplemental technique to traditional genome analysis such as conventional meiotic pairing analysis.

Rapid developments in genetics, molecular genetics, molecular biology, and genomics, together with molecular cytogenetics, have driven major conceptual advances in mitosis, meiosis, chromosome structure, and chromosome

manipulation. People now realise that chromosome structure and function determine gene regulation, expression, and silencing. Cytogenetics has now become an integral part of genome analysis.

Wheat genetic stocks developed using cytogenetics techniques

Because of polyploidy, the wheat genome is highly buffered and can tolerate a high degree of aneuploidy, especially compared to diploid species. Using traditional cytogenetic techniques, E.R. Sears developed a series of unique and valuable cytogenetic stocks (Sears 1954, 1966*a*), which are still used widely and are very important for both cytogenetic and genetic studies in wheat. These stocks are a treasure for modern wheat cytogenetics. The great benefit of these aneuploids is that they provide cytogenetic markers for each of the 21 chromosomes and most of the 42 chromosome arms. Among these stocks, the most important and widely utilised stocks include nullisomic–tetrasomic (NT), monosomic, ditelosomic (Dt), and double ditelosomic (dDt) lines. Because of the NT lines, Sears (1966*a*) was able to place the 21 wheat chromosomes into 3 genomes and 7 homeologous groups. Monosomic and telosomic lines allowed researchers to locate genes and DNA markers to individual chromosomes (McIntosh *et al*. 1995) and chromosome arms (Sears 1966*b*). At present, the ditelosomic stocks are being used for flow-sorting and constructing chromosome arm specific BAC libraries (Vrána *et al*. 2000; Doležel *et al*. 2003; Šafář *et al*. 2004), which are crucial for sequencing the gene-rich regions of the individual chromosome arms (Gill *et al*. 2004). Thus, much of cytogenetic work by Sears revolutionised the study of wheat genetics and genomics.

Endo and Gill (1996) isolated more than 400 deletion stocks involving all 42 arms of wheat using the action of a gametocidal (*Gc*) gene (Endo 1978, 1990) combined with chromosome banding. These deletion stocks, with various sized terminal deletions in individual chromosome arms, are useful for the targeted physical mapping of any gene or DNA sequence of interest to a defined chromosome bin and, therefore, provide a unique way of conducting physical mapping in wheat (Faris *et al*. 2000). Cytogenetically based physical maps for all 7 homeologous groups of wheat have been constructed with the help of the deletion stocks (Hohmann *et al*. 1994; Delaney *et al*. 1995*a*, 1995*b*; Mickelson-Young *et al*. 1995; Gill *et al*. 1996*a*, 1996*b*; Roder *et al*. 1998; Weng *et al*. 2000). These deletion lines are currently a critical and powerful resource for wheat genome mapping projects, such as the EST mapping project funded by the National Science Foundation in the United States (Qi *et al*. 2004). This mapping project bin-mapped over 16 000 EST loci, providing insights on micro-colinearity with rice and fundamentals for comparative mapping with rice and *Arabidopsis* (Conley *et al*. 2004; Linkiewicz *et al*. 2004; Munkvold *et al*. 2004; Peng *et al*. 2004). In addition, the deletion stocks were crucial in relating genetic maps to physical maps of chromosomes, map-based cloning of genes (Feuillet *et al*. 2003; Huang *et al*. 2003; Yan *et al*. 2003, 2004; Simons *et al*. 2006), and studying the distribution of genes (Gill *et al*. 1996*a*) and recombination frequency along the chromosomes (Akhunov *et al*. 2003).

The power and utility of these cytogenetic stocks and those being developed by present cytogeneticists are even more realised when combined with molecular technology. The significance of these stocks as tools for wheat genetics and genomics studies cannot be overestimated.

Study of chromosome landmarks using cytogenetic techniques

Cytogenetic techniques are excellent tools to study the structure and function of chromosome landmarks, such as heterochromatin, rRNA genes (Mukai *et al*. 1990; Jiang and Gill 1994*b*), the centromere (Zhang *et al*. 2004*a*), and the telomere and subtelomere (Zhang *et al*. 2004*a*). Physical locations of various repetitive DNA sequences can be used to analyse the molecular nature of heterochromatin (Bedbrook *et al*. 1980; Appels *et al*. 1981).

Centromere

The centromere is a cytologically visible component of a chromosome appearing as a primary constriction at metaphase. It plays an essential role in the accurate segregation of chromosomes during mitosis and meiosis. In recent years, several centromere-associated repetitive sequences have been characterised and mapped to the centromeric regions of chromosomes of grass species by FISH (Aragon-Alcaide *et al*. 1996; Jiang *et al*. 1996; Dong *et al*. 1998; Presting *et al*. 1998; Francki 2001; Zhang *et al*. 2004*a*). Among these sequences, only 2 are species-specific, i.e. rye (Francki 2001) and sorghum (Miller *et al*. 1998). All the others are common to many grass species, including rice, maize, sorghum, rye, barley, and wheat. The presence of these centromere-specific repetitive sequences in different members of the Gramineae indicates that the cereal centromere may have evolved from a common progenitor before divergence about 60 million years ago (Kumar and Bennetzen 1999). Although the function of these sequences remains unknown, they may be related to centromere function because of their location and high degree of repetition.

Using a common grass centromeric probe pRCS1 (Dong *et al*. 1998) (Fig. 1*a*) and a rye-specific centromeric probe pAWRC.1 (Francki 2001) (Fig. 1*b*) in FISH experiments, Zhang *et al*. (2001) demonstrated for the first time the compound structure of the centromere and the hybrid nature of centromeres in wheat–rye translocation chromosomes, indicating that centric breakage–fusion can occur at different positions within the primary constriction without influencing the centromere function and behaviour. Probe pAWRC.1 was also used to physically map the centromeric breakpoints and to characterise breakpoints in wheat–rye translocation lines, which have been stably maintained in breeding materials (Francki *et al*. 2001).

Subtelomere and telomere

Subtelomeres are extraordinarily dynamic and variable regions near the ends of chromosomes. They are defined by their unusual structure, patchworks of repeat blocks that are duplicated. A subtelomeric repeat identified and isolated from *Aegilops tauschii* (Zhang *et al*. 2004*a*) hybridised to all subtelomeric chromosome regions in wheat, *Aegilops* species, rye, barley, and oat. This subtelomeric tandem repeat is present with high copy

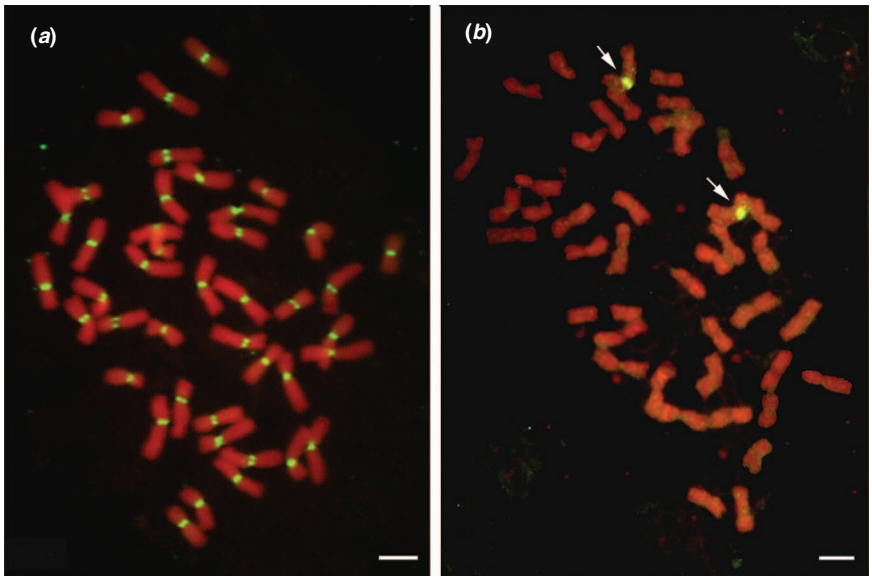

Fig. 1. Fluorescence *in situ* hybridisation (FISH) pattern of (*a*) a common grass centromeric probe pRCS1 and (*b*) a rye-specific centromeric probe pAWRC.1 on mitotic metaphase chromosomes of *Triticum aestivum* cv. Chinese Spring (CS) (*a*) and a wheat–rye addition line (*b*). Probe DNAs were labelled with biotin-14-dATP and detected with fluorescein-avidin DN, which was visualised by yellow-green fluorescence. Chromosomes were counterstained with propidium iodide and fluoresced red. (*a*) FISH pattern of probe pRCS1, which hybridised strongly to the centromeric regions of all the chromosomes in *T. aestivum*; (*b*) probe pAWRC.1 hybridised only to the centromeres of the rye chromosomes in this wheat–rye addition line and not to the centromeres of the wheat chromosomes. Arrows point to the centromeres of chromosome 6R. Bars represent 10 m.

numbers in the above species, indicating that it is common within the Triticeae. The presence of similar subtelomeric sequences in different species indicates an ancient origin, because if the subtelomeric sequences are species- or genome-specific they presumably evolved more recently (Vershinin *et al*. 1995).

The complex and variable nature of subtelomeres has made it difficult to assess the possible function of these regions. However, because of the abundance of subtelomeric sequences in all chromosome arms in the above Triticeae species, we know that they must have had some important function(s) during evolution.

Telomeres define the ends of the chromosomes and protect the ends of chromosomes from degradation and end-to-end fusion. The maintenance of telomere length is crucial for chromosome stability and integrity and for cell survival in eukaryotes. In wheat, newly broken ends of chromosomes were healed by *de novo* addition of telomeric repeats as analysed by FISH (Friebe *et al*. 2001). In order to examine whether subtelomeric repeats are also vital for the stability of chromosomes, and hence indispensable, several wheat deletion lines were analysed by FISH using both subtelomeric (pAet7-L3) (Zhang *et al*. 2004*a*) and telomeric repeats (pAtT4) (Richards and Ausubel 1988) as probes. As seen in Fig. 2, the 1BL arm has telomeric repeats,

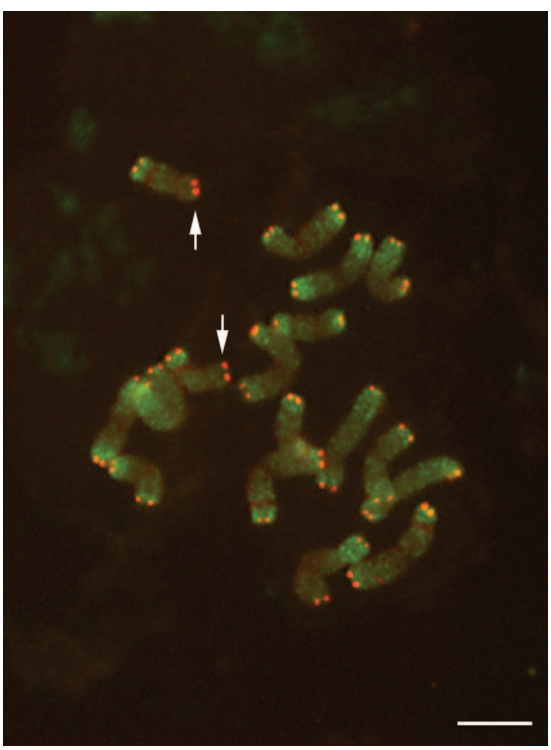

Fig. 2. FISH pattern of telomeric (pAtT4) and subtelomeric (pAet7-L3) probes on mitotic metaphase chromosomes of a CS deletion line 1BL-6. Approximately 68% of the distal portion of the 1B long arm was deleted. The telomeric probe was labelled with tetramethyl-rhodamine-5-dUTP and fluoresced red. It hybridised to the telomeres of all chromosomes. The subtelomeric probe was labelled with biotin-14-dATP and detected with fluorescein-avidin DN, which was visualised by green fluorescence. It hybridised to all subtelomeric chromosome regions except the deleted 1BL (arrows). Bar represent 10 m.

but not subtelomeric repeats, indicating that only telomeric repeats were added to the chromosome end after deletion (P. Zhang, B. Friebe, B. S. Gill, unpublished data). Because these deletion lines behave normally in mitosis and meiosis, it seems that the absence of subtelomeric repeats does not influence the function of the chromosomes. Similarly, mutations in *Plasmodium falciparum* that resulted in the deletion of subtelomeric sequences indicated that subtelomeres are not required for the viability of an organism, nor for proper chromosome segregation at mitosis or meiosis (Pologe and Ravetch 1988). So far, no cases have been reported where all subtelomeric repeats were removed from an organism. Therefore, some subtelomeric repeats are probably required for viability.

The role of cytogenetics in breeding for disease resistance in cereals

Hexaploid wheat evolved through 2 natural interspecific hybridisations and chromosome doublings. In addition, domestication via human selection for desirable traits such as free threshability have taken place. Furthermore, no wild hexaploid wheat species exist. Therefore, throughout its entire existence wheat has been a genetically narrow species with relatively low genetic diversity compared to its wild relatives, which have had a much longer time to evolve and adapt to the natural environment. The surviving genotypes of wild species often carry resistance genes for harsh conditions, such as biotic (e.g. diseases and pests) and abiotic stresses (e.g. heat and cold, drought, and salinity), and thus are important reservoirs of genetic diversity for common wheat.

Under modern agricultural systems, popular wheat cultivars may be planted over wide areas because of desirable agronomic traits such as high yield or superior quality. Their relatively limited genetic diversity makes them vulnerable to new races of pathogens and insects, which are continually evolving in response to their environment. In order to reduce this vulnerability and broaden the genetic diversity in cultivated wheat, many agronomically important genes, including disease resistance genes, have been introgressed into wheat from alien species by taking the advantage of the crossability of wheat with its related species (McIntosh 1991; Jiang *et al*. 1994; McIntosh *et al*. 1995; Friebe *et al*. 1996).

The first alien resistance gene transferred into wheat was *Lr9*. Radiation treatment of pollen was used to induce chromosome breakage in order to recombine the alien chromatin with that of wheat (Sears 1956). Much later, C-banding and GISH patterns indicated that the chromosomes involved in the *Lr9* transfer were derived from *Ae. umbellulata* (Friebe *et al*. 1996). Since the 1950s, cytogenetic tools have been applied to identify new sources of disease resistance (such as resistance to wheat streak mosaic virus, barley yellow dwarf virus, powdery mildew, and rusts) and to introgress resistance genes from alien species into wheat. For example, 23 catalogued leaf rust, 19 stem rust, and 6 stripe rust resistance genes have been transferred into wheat from the primary, secondary, and tertiary gene pools (Sharma and Gill 1983; Jiang *et al*. 1994; McIntosh *et al*. 1995, 2003; Friebe *et al*. 1996). Several of these genes have been exploited in cultivar improvement and some are still effective in at least some agricultural regions (McIntosh *et al*. 1995).

Monitoring alien chromatin during introgression is critical for a successful transfer. In the past, traditional cytogenetic methods, including meiotic chromosome pairing, monosomic analysis, and telocentric mapping, were used to characterise the products of wide crosses. Today, state-of-the-art cytogenetic techniques such as C-banding (Gill and Kimber 1974*a*, 1974*b*; Lukaszewski and Gustafson 1983; Friebe and Larter 1988; Gill *et al*. 1991) and GISH (Le *et al*. 1989; Friebe *et al*. 1992) are used routinely because they are the most efficient techniques to directly and precisely detect the alien segment in wheat. C-banding allows identification of the wheat and alien chromosomes involved in the translocations provided the alien segments have diagnostic bands, whereas GISH allows breakpoints to be localised and an estimation of the amount of alien chromatin present in translocation chromosomes. These 2 techniques, together with various molecular markers, proved useful for detecting alien segments in wheat backgrounds.

GISH is excellent in differentiating chromosomes of species that are not closely related by homology. However, distinguishing species that have close affinities to each other, such as the A-, B-, and D-genomes (or their diploid progenitors) in wheat, is difficult (Mukai *et al*. 1993). Two genome-specific dispersed repeats (A- and D-genome) in wheat were identified and isolated using FISH and shotgun subcloning techniques (Zhang *et al*. 2004*b*), providing an easier and more reliable technique compared to GISH for simultaneously differentiating the A-, B-, and D-genome chromosomes and detecting intergenomic translocations involving the A- and/or D-genome chromosomes in wheat.

The approach used to produce wheat–alien translocations for transferring alien target genes into the wheat genome depends on many factors, the most important of which are the chromosomal location of the gene and whether or not the alien chromosome carrying the gene has synteny with the recipient wheat chromosome.

The first approach is radiation treatment. Although radiation treatment was used in the past to transfer alien genes into wheat (Sears 1956; Knott 1961; Sharma and Knott 1966), it is not preferred because the random chromosome breakage caused by radiation produces translocations that are non-compensating (Friebe *et al*. 1993, 1996) and genetically unbalanced, leading to reduced agronomic performance, and thus preventing their application in cultivar or germplasm improvement. In addition, radiation treatment may cause additional chromosome aberrations in the wheat genome.

The second group of approaches includes tissue culture and spontaneous translocation. Chromosomal translocations can happen spontaneously or during tissue culture (Lapitan *et al*. 1984). They can be either centric breakage–fusion products, which are whole-arm translocations such as the 1BL.1RS translocation (Mettin *et al*. 1973; Zeller 1973), or non-centric breakage–fusion products in which the breakpoints can be anywhere in the chromosome but not in the centromere. However, because of their low frequency and non-compensating nature, problems exist when wheat–alien chromosome translocations happen spontaneously or by tissue

culture. Even though they have been used in the past to produce some very successful transfers (Smith *et al*. 1968; Mettin *et al*. 1973; Zeller 1973), they are not the preferred methods for producing wheat–alien chromosome translocations. Nevertheless, if the target alien gene is located in the proximal region of the chromosome where recombination is generally suppressed, or the alien or recipient wheat chromosomes are structurally modified so that the synteny is not conserved, the above 2 approaches are the only choices. However, they need to be accompanied by strong selection for the recovery of compensating translocations in order to be successful (Sears 1993).

The third approach involves univalent misdivision and induced homeologous recombination. The majority of genes are located in the distal regions of chromosomes where recombination is much more frequent than the proximal region. If the gene synteny is conserved and the recipient wheat chromosome does not carry important fertility or other pivotal genes, then the following procedures can be used for the direct transfer of alien genes from a non-homologous chromosome of a wild species from the secondary (where the target gene is not located on a homologous chromosome) or tertiary gene pools into wheat and avoid non-compensating translocations. First, wheat is hybridised with the alien species, followed by the production of amphiploids and backcrossing. Disomic alien chromosome addition and double monosomic substitution lines can then be produced. The next step is to produce compensating wheat–alien Robertsonian translocations, taking advantage of the centric breakage–fusion mechanism of univalents at meiotic metaphase I (Sears 1952). These compensating translocations are agronomically desirable and may have agronomic potential in cultivar improvement. Once whole-arm Robertsonian translocation lines are developed, homeologous recombination can be induced to reduce the size of the alien segments or to recombine genes from different parents into one line using either the *ph1b* mutant (Sears 1981) or suppressing the effect of the *Ph1* gene (Riley *et al*. 1968). This manipulation is needed because normally linkage between useful resistance genes and undesirable or deleterious alien genes will influence negatively the end-use quality (Knott 1968, 1989) and grain yield (The *et al*. 1988). Because alien chromosomes normally do not recombine with those of wheat, chromosome engineering is required to break the linkage drag, reduce the amount of alien material, and transfer only the beneficial resistance genes to wheat. Later, proximal and distal primary recombinants can be intercrossed to generate secondary recombinants with small interstitial translocations including the gene of interest from the alien species (Sears 1983; Lukaszewski 2000; Faris *et al*. 2002; Zhang *et al*. 2005; Dundas *et al*. 2007; M. Ferrahi, B. Friebe, B. S. Gill, unpublished data). Despite their alien origins, disease resistance genes introgressed from alien species have not generally proved to be durable. Each gene must be considered a routine addition to the overall pool of resistance genes available in wheat. On the other hand, single major resistance genes normally are not durable because they can be easily broken down by the mutated pathogens. Therefore, resistance gene combinations/pyramiding using cytogenetics and molecular biological techniques are highly desirable. In addition, the enormous range of newly developed genetic and genomic resources, fast advancing molecular tools, and high throughput genotyping approaches are extremely increasingly useful in development of improved cereal cultivars.

Concluding remarks

Cytogenetics is an integral part of genome analysis. We still need cytogenetics in the age of molecular genetics. The large genome size and high percentage of repetitive DNA sequences in wheat make molecular genetic studies difficult. However, these characteristics make wheat amenable to cytogenetic studies. Cytogenetics has its own niche and complements molecular genetics analysis. Considerable progress has been made in alien gene transfer into wheat with help from cytogenetic techniques in almost every step. The role of cytogenetics in identifying new disease resistance sources, developing resistant germplasm, and breeding for durable resistance to different diseases in cereals, especially in wheat, cannot be replaced by any other technique. While genetic engineering offers opportunities for the future, the problems of gene identification, gene cloning, and social acceptance of engineered derivatives are still to be solved.

Acknowledgments

This research was supported by Grains Research and Development Corporation, the Kansas Wheat Commission, and a special USDA grant to the Wheat Genetics Resource Center. We thank W. John Raupp, Duane Wilson, and Sami Hoxha for their excellent assistance; and Prof. Robert McIntosh and Dr Harbans Bariana for beneficial discussion.

References

Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, *et al.* (2000) The genome sequence of *Drosophila melanogaster. Science* **287**, 2185–2195. doi: 10.1126/science.287.5461.2185

Akhunov ED, Goodyear AW, Geng S, Qi L, Echalier B, *et al.* (2003) The organization and rate of evolution of wheat genomes are correlated with recombination rates along chromosome arms. *Genome Research* **13**, 753–763. doi: 10.1101/gr.808603

Appels R, Dennis ES, Smyth DR, Peacock WJ (1981) Two repeated DNA sequences from the heterochromatic regions of rye (*Secale cereale*) chromosomes. *Chromosoma* **84**, 265–277. doi: 10.1007/BF00399137

Aragon-Alcaide L, Miller T, Schwarzacher T, Reader S, Moore G (1996) A cereal centromeric sequence. *Chromosoma* **105**, 261–268.

Bedbrook JR, Jones J, O'Dell M, Thompson RD, Flavell RB (1980) A molecular description of telomeric heterochromatin in *Secale* species. *Cell* **19**, 545–560. doi: 10.1016/0092-8674(80)90529-2

Boveri T (1902) Über mehrpolige Mitosen als Mittel zur Analyse des Zellkerns. Verhandl. *Deut. Physiol. Med. Gesellsch. zur Würzburg* **35**, 67–90.

Conley EJ, Nduati V, Gonzalez-Hernandez JL, Mesfin A, Trudeau-Spanjers M, *et al.* (2004) A 2600-locus chromosome bin map of wheat homoeologous group 2 reveals interstitial gene-rich islands and colinearity with rice. *Genetics* **168**, 625–637. doi: 10.1534/genetics.104.034801

Delaney D, Nasuda S, Endo TR, Gill BS, Hulbert SH (1995*a*) Cytogenetically based physical map of the group-2 chromosomes of wheat. *Theoretical and Applied Genetics* **91**, 568–573.

Delaney D, Nasuda S, Endo TR, Gill BS, Hulbert SH (1995*b*) Cytogenetically based physical map of the group-3 chromosomes of wheat. *Theoretical and Applied Genetics* **91**, 780–782.

Doležel J, Šafář J, Janda J, Bartoš J, Kubaláková M, Číhalíková J, Šimková H, Sourdille P, Bernard M, Chalhoub B (2003) Development of flow cytogenetics for wheat genome mapping. In 'Proceedings of the 10th International Wheat Genetics Symposium'. (Eds NE Pogna, M Romano, EA Pogna, G Galterio) pp. 65–68. (Paestum: Italy)

Dong F, Miller T, Jackson SA, Wang GL, Ronald PC, Jiang J (1998) Rice (*Oryza sativa*) centromeric regions consist of complex DNA. *Proceedings of the National Academy of Sciences of the United States of America* **95**, 8135–8140. doi: 10.1073/pnas.95.14.8135

Dundas IS, Anugrahwati DR, Verlin DC, Park RF, Bariana HS, Mago R, Islam AKMR (2007) New sources of rust resistance from alien species: meliorating linked defects and discovery. *Australian Journal of Agricultural Research* **58**, 545–549.

Dunham I, Shimizu N, Roe BA, Chissoe S, Hunt AR, *et al.* (1999) The DNA sequence of human chromosome 22. *Nature* **402**, 489–495. doi: 10.1038/990031

Eisenstein M (2005) A look back: FISH still fresh after 25 years. *Nature Methods* **2**, 236. doi: 10.1038/nmeth0305-236

Endo TR (1978) On the *Aegilops* chromosomes having gametocidal action on common wheat. In 'Proceedings of the 5th International Wheat Genetics Symposium'. (Ed. S Ramanujan) pp. 306–314. (Indian Society of Genetics and Plant Breeding: New Delhi)

Endo TR (1990) Gametocidal chromosomes and their induction of chromosome mutations in wheat. *Japanese Journal of Genetics* **65**, 135–152. doi: 10.1266/jjg.65.135

Endo TR, Gill BS (1996) The deletion stocks of common wheat. *Journal of Heredity* **87**, 295–307.

Faris JD, Friebe B, Gill BS (2002) Wheat genomics: Exploring the polyploidy model. *Current Genomics* **3**, 577–591. doi: 10.2174/1389202023350219

Faris JD, Haen KM, Gill BS (2000) Saturation mapping of a gene-rich recombination hot spot region in wheat. *Genetics* **154**, 823–835.

Feuillet C, Travella S, Stein N, Albar L, Nublat A, Keller B (2003) Map-based isolation of the leaf rust disease resistance gene *Lr10* from the hexaploid wheat (*Triticum aestivum* L.) genome. *Proceedings of the National Academy of Sciences of the United States of America* **100**, 15253–15258. doi: 10.1073/pnas.2435133100

Francki MG (2001) Identification of *Bilby*, a diverged centromeric Ty1-*copia* retrotransposon family from cereal rye (*Secale cereale* L.). *Genome* **44**, 266–274. doi: 10.1139/gen-44-2-266

Francki MG, Berzonsky WA, Ohm HW, Anderson JM (2001) Physical location of a *HSP70* homologue on the centromere of chromosome 1B of wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics* **104**, 184–191.

Fransz PF, Alonso-Blanco C, Liharska TB, Peeters AJM, Zabel P, de Jong JH (1996) High-resolution physical mapping in *Arabidopsis thaliana* and tomato by fluorescence in situ hybridisation to extended DNA fibres. *The Plant Journal* **9**, 421–430. doi: 10.1046/j.1365-313X.1996.09030421.x

Friebe B, Jiang J, Gill BS, Dyck PL (1993) Radiation-induced nonhomoeologous wheat-*Agropyron intermedium* chromosomal translocations conferring resistance to leaf rust. *Theoretical and Applied Genetics* **86**, 141–149. doi: 10.1007/BF00222072

Friebe B, Jiang J, Raupp WJ, McIntosh RA, Gill BS (1996) Characterization of wheat-alien translocations conferring resistance to diseases and pests: current status. *Euphytica* **91**, 59–87.

Friebe B, Kynast RG, Zhang P, Qi L, Dhar M, Gill BS (2001) Chromosome healing by addition of telomeric repeats in wheat occurs during the first mitotic divisions of the sporophyte and is a gradual process. *Chromosome Research* **9**, 137–146. doi: 10.1023/A:1009283003903

Friebe B, Larter EN (1988) Identification of a complete set of isogenic wheat/rye D genome substitution lines by means of Giemsa C-banding. *Theoretical and Applied Genetics* **76**, 473–479. doi: 10.1007/BF00265353

Friebe B, Zeller FJ, Mukai Y, Forster BP, Bartos P, McIntosh RA (1992) Characterization of rust resistant wheat-*Agropyron intermedium* derivatives by C-banding, in situ hybridization and isozyme analysis. *Theoretical and Applied Genetics* **83**, 775–782.

Gall JG, Pardue ML (1969) Formation and detection of RNA-DNA hybrid molecules in cytological preparations. *Proceedings of the National Academy of Sciences of the United States of America* **63**, 378–383. doi: 10.1073/pnas.63.2.378

Gill BS, Appels R, Botha-Oberholster A-M, Buell CR, Bennetzen JL, *et al.* (2004) A workshop report on wheat genome sequencing: International genome research on wheat consortium. *Genetics* **168**, 1087–1096. doi: 10.1534/genetics.104.034769

Gill BS, Friebe B, Endo TR (1991) Standard karyotype and nomenclature system for description of chromosome bands and structural aberrations in wheat (*Triticum aestivum*). *Genome* **34**, 830–839.

Gill KS, Gill BS, Endo TR, Boiko EV (1996*a*) Identification and high-density mapping of gene-rich regions in chromosome group 5 of wheat. *Genetics* **143**, 1001–1012.

Gill KS, Gill BS, Endo TR, Taylor T (1996*b*) Identification and high-density mapping of gene-rich regions in chromosome group 1 of wheat. *Genetics* **144**, 1883–1891.

Gill BS, Kimber G (1974*a*) The giemsa C-banded karyotype of rye. *Proceedings of the National Academy of Sciences of the United States of America* **71**, 1247–1249. doi: 10.1073/pnas.71.4.1247

Gill BS, Kimber G (1974*b*) Giemsa C-banding and the evolution of wheat. *Proceedings of the National Academy of Sciences of the United States of America* **71**, 4086–4090. doi: 10.1073/pnas.71.10.4086

Hohmann U, Endo TR, Gill KS, Gill BS (1994) Comparison of genetic and physical maps of group 7 chromosomes from *Triticum aestivum* L. *Molecular Genetics and Genomics* **245**, 644–653.

Huang L, Brooks SA, Li W, Fellers JP, Trick HN, Gill BS (2003) Map-based cloning of a rust-resistance gene from bread wheat's large polyploid genome. *Genetics* **164**, 655–664.

Jackson SA, Wang ML, Goodman HM, Jiang J (1998) Application of fiber-FISH in physical mapping of *Arabidopsis thaliana. Genome* **41**, 566–572. doi: 10.1139/gen-41-4-566

Jiang J, Friebe B, Gill BS (1994) Recent advances in alien gene transfer in wheat. *Euphytica* **73**, 199–212. doi: 10.1007/BF00036700

Jiang J, Gill BS (1994*a*) Nonisotopic in situ hybridization and plant genome mapping: the first 10 years. *Genome* **37**, 717–725.

Jiang J, Gill BS (1994*b*) New 18S–26S ribosomal RNA gene loci: chromosomal landmarks for the evolution of polyploid wheats. *Chromosoma* **103**, 179–185.

Jiang J, Gill BS (1994*c*) Different species-specific chromosome translocations in *Triticum timopheevii* and *T. turgidum* support the diphyletic origin of polyploid wheats. *Chromosome Research* **2**, 59–64. doi: 10.1007/BF01539455

Jiang J, Nasuda S, Dong F, Scherrer CW, Woo SS, Wing RA, Gill BS, Ward DC (1996) A conserved repetitive DNA element located in the centromeres of cereal chromosomes. *Proceedings of the National Academy of Sciences of the United States of America* **93**, 14210–14213. doi: 10.1073/pnas.93.24.14210

Knott DR (1961) The inheritance of rust resistance. VI. The transfer of stem rust resistance from *Agropyron elongatum* to common wheat. *Canadian Journal of Plant Science* **41**, 109–123.

Knott DR (1968) Translocations involving *Triticum* chromosomes and *Agropyron* chromosomes carrying rust resistance. *Canadian Journal of Genetics and Cytology* **10**, 695–696.

Knott DR (1989) The effect of transfers of alien genes for leaf rust resistance on the agronomic and quality characteristics of wheat. *Euphytica* **44**, 65–72. doi: 10.1007/BF00022601

Kumar A, Bennetzen JL (1999) Plant retrotransposons. *Annual Review of Genetics* **33**, 479–532. doi: 10.1146/annurev.genet.33.1.479

Langer-Safer PR, Levine M, Ward DC (1982) Immunological method for mapping genes on *Drosophila* polytene chromosomes. *Proceedings of the National Academy of Sciences of the United States of America* **79**, 4381–4385. doi: 10.1073/pnas.79.14.4381

Lapitan NLV, Sears EG, Gill BS (1984) Translocations and other karyotypic structural changes in wheat × rye hybrids regenerated from tissue culture. *Theoretical and Applied Genetics* **68**, 547–554. doi: 10.1007/BF00285012

Le HT, Armstrong KC, Miki B (1989) Detection of rye DNA in wheat-rye hybrids and wheat translocation stocks using total genomic DNA as a probe. *Plant Molecular Biology Reporter* **7**, 150–158.

Leitch IJ, Heslop-Harrison JS (1993) Physical mapping of four sites of 5S rDNA sequences and one site of the α-amylase-2 gene in barley (*Hordeum vulgare*). *Genome* **36**, 517–523.

Linkiewicz AM, Qi LL, Gill BS, Ratnasiri A, Echalier B, *et al.* (2004) A 2500-locus bin map of wheat homoeologous group 5 provides insights on gene distribution and colinearity with rice. *Genetics* **168**, 665–676. doi: 10.1534/genetics.104.034835

Lukaszewski AJ (2000) Manipulation of the 1RS.1BL translocation in wheat by induced homoeologous recombination. *Crop Science* **40**, 216–225.

Lukaszewski AJ, Gustafson JP (1983) Translocations and modifications of chromosomes in triticale × wheat hybrids. *Theoretical and Applied Genetics* **64**, 239–248. doi: 10.1007/BF00303771

McIntosh RA (1991) Alien sources of disease resistance in bread wheats. In 'Proceedings of Dr. H. Kihara Memorial International Symposium on Cytoplasmic Engineering in Wheat. Nuclear and Organellar Genomes of Wheat Species'. (Eds T Sasakuma, T Kinoshita) pp. 320–332.

McIntosh RA, Wellings CR, Park RF (1995) 'Wheat rusts. An atlas of resistance genes.' (CSIRO Publishing: Melbourne)

McIntosh RA, Yamazaki Y, Devos KM, Dubcovsky J, Rogers WJ, Appels R (2003) Catalogue of gene symbols for wheat. In 'Proceedings of 10th International Wheat Genetics Symposium'. Vol. 4. (Eds NE Pogna, M Romanò, EA Pogna, G Galterio) (Paestum: Italy)

Mettin D, Bluthner WD, Schlegel G (1973) Additional evidence on spontaneous 1B/1R wheat-rye substitutions and translocations. In 'Proceedings of 4th International Wheat Genetics Symposium'. Columbia, Missouri (Eds ER Sears, LMS Sears) pp. 179–184.

Mickelson-Young L, Endo TR, Gill BS (1995) A cytogenetic ladder-map of wheat homoeologous group-4 chromosomes. *Theoretical and Applied Genetics* **90**, 1007–1011. doi: 10.1007/BF00222914

Miller JT, Jackson SA, Nasuda S, Gill BS, Wing RA, Jiang J (1998) Cloning and characterization of a centromere-specific repetitive DNA element from *Sorghum bicolor. Theoretical and Applied Genetics* **96**, 832–839. doi: 10.1007/s001220050809

Morgan TH, Sturtevant AH, Muller HJ, Bridges CB (1915) 'The mechanism of Mendelian heredity.' (Henry Holt and Co.: New York)

Mukai Y, Endo TR, Gill BS (1990) Physical mapping of the 5S rRNA multigene family in common wheat. *Journal of Heredity* **81**, 290–295.

Mukai Y, Nakahara Y, Yamamoto M (1993) Simultaneous discrimination of the three genomes in hexaploid wheat by multicolor fluorescence in situ hybridization using total genomic and highly repeated DNA probes. *Genome* **36**, 489–494.

Munkvold JD, Greene RA, Bermudez-Kandianis CE, La Rota CM, Edwards H, *et al.* (2004) Group 3 chromosome bin maps of wheat and their relationship to rice chromosome 1. *Genetics* **168**, 639–650. doi: 10.1534/genetics.104.034819

Naranjo T, Roca A, Goicoechea PG, Giraldez R (1987) Arm homoeology of wheat and rye chromosomes. *Genome* **29**, 873–882.

Painter TS (1933) A new method for the study of chromosome rearrangements and the plotting of chromosome maps. *Science* **78**, 585–586. doi: 10.1126/science.78.2034.585

Pedersen C, Langridge P (1997) Identification of the entire chromosome complement of bread wheat by two-color FISH. *Genome* **40**, 589–593.

Peng JH, Zadeh H, Lazo GR, Gustafson JP, Chao S, *et al.* (2004) Chromosome bin map of expressed sequence tags in homoeologous group 1 of hexaploid wheat and homoeology with rice and *Arabidopsis*. *Genetics* **168**, 609–623. doi: 10.1534/genetics.104.034793

Pinkel D, Straume T, Gray JW (1986) Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. *Proceedings of the National Academy of Sciences of the United States of America* **83**, 2934–2938. doi: 10.1073/pnas.83.9.2934

Pologe LG, Ravetch JV (1988) Large deletions result from breakage and healing of *P. falciparum* chromosomes. *Cell* **55**, 869–874. doi: 10.1016/0092-8674(88)90142-0

Presting GG, Malysheva L, Fuchs J, Schubert I (1998) A Ty3/*gypsy* retrotransposon-like sequence localizes to the centromeric regions of cereal chromosomes. *The Plant Journal* **16**, 721–728. doi: 10.1046/j.1365-313x.1998.00341.x

Qi LL, Echalier B, Chao S, Lazo GR, Butler GE, *et al.* (2004) A chromosome bin map of 16 000 expressed sequence tag loci and distribution of genes among the three genomes of polyploid wheat. *Genetics* **168**, 701–712. doi: 10.1534/genetics.104.034868

Rayburn AL, Gill BS (1985) Use of biotin-labeled probes to map specific DNA sequences on wheat chromosomes. *Journal of Heredity* **76**, 78–81.

Richards EJ, Ausubel FM (1988) Isolation of a higher eukaryotic telomere from *Arabidopsis thaliana. Cell* **53**, 127–136. doi: 10.1016/0092-8674(88)90494-1

Riley R, Chapman V, Johnson R (1968) Introduction of yellow-rust resistance of *Aegilops comosa* into wheat by genetically induced homoeologous recombination. *Nature* **217**, 383–384. doi: 10.1038/217383a0

Roder MS, Korzun V, Wendehake K, Plaschke J, Tixier M-H, Leroy P, Ganal MW (1998) A microsatellite map of wheat. *Genetics* **149**, 2007–2023.

Šafář J, Bartoš J, Janda J, Bellec A, Kubaláková M, *et al.* (2004) Dissecting large and complex genomes: flow sorting and BAC cloning of individual chromosomes from bread wheat. *The Plant Journal* **39**, 960–968. doi: 10.1111/j.1365-313X.2004.02179.x

Schmidt T, Heslop-Harrison JS (1996) High resolution mapping of repetitive DNA by in situ hybridization: Molecular and chromosomal features of prominent dispersed and discretely localized DNA families from the wild beet species *Beta procumbens. Plant Molecular Biology* **30**, 1099–1114. doi: 10.1007/BF00019545

Sears ER (1952) Misdivision of univalents in common wheat. *Chromosoma* **4**, 535–550. doi: 10.1007/BF00325789

Sears ER (1954) The aneuploids of common wheat. *Research Bulletin University of Missouri Agricultural Experiment Station* **572**, 1–58.

Sears ER (1956) The transfer of leaf rust resistance from *Aegilops umbellulata* to wheat. *Brookhaven Symposia in Biology* **9**, 1–22.

Sears ER (1966*a*) Nullisomic-tetrasomic combinations in hexaploid wheat. In 'Chromosome Manipulations and Plant Genetics. 10th International Botanical Congress'. (Eds R Riley, KR Lewis) pp. 29–45. (Plenum Press: New York)

Sears ER (1966*b*) Chromosome mapping with the aid of telocentrics. In 'Proceedings of the 2nd International Wheat Genetics Symposium'. *Hereditas* **2**(Suppl.), 370–381.

Sears ER (1981) Transfer of alien genetic material to wheat. In 'Wheat science—today and tomorrow'. (Eds LT Evans, WJ Peacock) pp. 75–89. (Cambridge University Press: Cambridge, UK)

Sears ER (1983) The transfer to wheat of interstitial segment of alien chromosomes. In 'Proceedings of the 6th International Wheat Genetics Symposium'. (Ed. S Sakamoto) pp. 5–12.

Sears ER (1993) Use of radiation to transfer alien segments to wheat. *Crop Science* **33**, 897–901.

Sharma D, Knott DR (1966) The transfer of leaf-rust resistance from *Agropyron* to *Triticum* by irradiation. *Canadian Journal of Genetics and Cytology* **8**, 137–143.

Sharma HC, Gill BS (1983) Current status of wide hybridization in wheat. *Euphytica* **32**, 17–31. doi: 10.1007/BF00036860

Simons KJ, Fellers JP, Trick HN, Zhang Z, Tai Y-S, Gill BS, Faris JD (2006) Molecular characterization of the major wheat domestication gene Q. *Genetics* **172**, 547–555. doi: 10.1534/genetics.105.044727

Smith EL, Schlehuber AM, Young HC Jr, Edwards LH (1968) Registration of Agent wheat. *Crop Science* **8**, 511–512.

Sutton WS (1903) The chromosomes in heredity. *The Biological Bulletin* **4**, 231–251. doi: 10.2307/1535741

The TT, Latter BDH, McIntosh RA, Ellison FW, Brennan PS, Fisher J, Hollamby GJ, Rathjen AJ, Wilson RE (1988) Grain yields of near-isogenic lines with added genes for stem rust resistance. In 'Proceedings of the 7th International Wheat Genetics Symposium'. (Eds TM Miller, RMD Koebner) (Institute of Plant Science Research: Cambridge, UK)

Vershinin AV, Schwarzacher T, Heslop-Harrison JS (1995) The large-scale genomic organization of repetitive DNA families at the telomeres of rye chromosomes. *The Plant Cell* **7**, 1823–1833. doi: 10.1105/tpc.7.11.1823

Viegas-Pequignot E, Berrard S, Brice A, Apiou F, Mallet J (1991) Localization of a 900-bp-long fragment of the human choline acetyltransferase gene to 10q11.2 by non-radioactive in situ hybridization. *Genomics* **9**, 210–212. doi: 10.1016/0888-7543(91)90242-7

Vrána J, Kubaláková M, Šimková H, Číhalíková J, Lysák MA, Doležel J (2000) Flow sorting of mitotic chromosomes in common wheat (*Triticum aestivum* L.). *Genetics* **156**, 2033–2041.

Watson JD, Crick FHC (1953) Molecular structure of nuclei acids. A structure for deoxyribose nuclei acid. *Nature* **171**, 737–738. doi: 10.1038/171737a0

Weng Y, Tuleen NA, Hart GE (2000) Extended physical maps and a consensus physical map of the homoeologous group-6 chromosomes of wheat (*Triticum aestivum* L. em Thell.). *Theoretical and Applied Genetics* **100**, 519–527.

Woo S-S, Jiang J, Gill BS, Paterson AH, Wing RA (1994) Construction and characterization of a bacterial artificial chromosome library of *Sorghum bicolor*. *Nucleic Acids Research* **22**, 4922–4931. doi: 10.1093/nar/22.23.4922

Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J (2003) Positional cloning of the wheat vernalization gene *VRN1*. *Proceedings of the National Academy of Sciences of the United States of America* **100**, 6263–6268. doi: 10.1073/pnas.0937399100

Yan L, Loukoianov A, Blechl A, Tranquilli G, Ramakrishna W, SanMiguel P, Bennetzen JL, Echenique V, Dubcovsky J (2004) The wheat *VRN2* gene is a flowering repressor down-regulated by vernalization. *Science* **303**, 1640–1644. doi: 10.1126/science.1094305

Zeller FJ (1973) 1B/1R wheat–rye chromosome substitutions and translocations. In 'Proceedings of 4th International Wheat Genetics Symposium'. Columbia, Missouri (Eds ER Sears, LMS Sears) pp. 209–221.

Zhang P, Friebe B, Lukaszewski AJ, Gill BS (2001) The centromere structure in Robertsonian wheat-rye translocation chromosomes indicates that centric breakage-fusion can occur at different positions within the primary constriction. *Chromosoma* **110**, 335–344.

Zhang P, Li W, Fellers J, Friebe B, Gill BS (2004*a*) BAC-FISH in wheat identifies chromosome landmarks consisting of different types of transposable elements. *Chromosoma* **112**, 288–299. doi: 10.1007/s00412-004-0273-9

Zhang P, Li W, Friebe B, Gill BS (2004*b*) Simultaneous painting of three genomes in hexaploid wheat by BAC-FISH. *Genome* **47**, 979–987. doi: 10.1139/g04-042

Zhang W, Lukaszewski AJ, Kolmer J, Soria MA, Goyal S, Dubcovsky J (2005) Molecular characterization of durum and common wheat recombinant lines carrying leaf rust resistance (*Lr19*) and yellow pigment (*Y*) genes from *Lophopyrum ponticum*. *Theoretical and Applied Genetics* **111**, 573–582. doi: 10.1007/s00122-005-2048-y

Manuscript received 9 February 2007, accepted 17 April 2007